Patients and Methods: Children aged 0–15 years with serologically confirmed TBE, hospitalized from January 1982 to October 1996 were included in the study. Sequelae were assessed at the discharge from the hospital, at six months and later if indicated.

Results: During 15 years 256 children with TBE were hospitalized. No deaths were recorded. In 24 (10%) children sequelae were present at the discharge from the hospital. At follow-up, 6 patients fully recovered, 5 showed mild, 9 moderate and 4 severe sequelae. TBE specific immunoglobulin was not used in patients who developed sequelae. Boys were affected more frequently. The severity of sequelae was strongly age related, and severe sequelae were observed only in patients over 5 years of age. It seems that the severity of the disease increased in the last years.

Conclusion: Contrary to common belief, severe forms of TBE with long lasting or permanent sequelae occur in childhood. Routine immunization in endemic areas is therefore strongly advocated.

P1394 Clinical Aspects of TBE in Lithuania

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Objective: To analyse and to present the epidemiological and clinical characteristics of tick-borne encephalitis (TBE) in Kaunas region in Lithuania.

Methods: There are presented all cases of adult patients that were treated for TBE in Kaunas Clinical Hospital of Infectious Diseases in the period of 17 years (1979–1995). Epidemiological and clinical characteristics and laboratory diagnosis confirmation were analyzed. The diagnoses of TBE cases were based on epidemiologic history, clinical picture, and detection of antibodies in the paired sera, using the hemagglutination inhibition test.

Results: In the period of 1979–1995 there were diagnosed 326 cases of TBE. 71.78% of the patients were able to recall the fact of tick bite that took place 1–4 weeks before the onset of the disease. Only 27.3% of the patients were sent in with suspicion of TBE. There were 55.55% of males and 44.45% of females, prevailing age – 20–60 years. Among the clinical manifestation of disease the headache (98.8%), nausea (77.7%), vomiting (54.68%), muscle aches (33.23%) and increased temperature (100%) were the most frequent. Only 29.62% of the patients came to the hospital up to the 5th day of manifestation. The majority of patients was admitted to the hospital in August, September and October (76.05%). It is important to point out the presence of meningoencephalitic (66%) and meningitic (20%) forms. The results of spinal fluid analysis revealed the view of seromeningitis. In hemagglutination – inhibition reaction there was a growth in antibodies titre in 90% of cases.

Conclusions: In Lithuania TBE is an actual problem that may be solved by vaccination of risk groups and individual persons.

P1395 Study of Arboviral Infections in Ukraine

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Objectives: Ecology of arboviruses, etiology and natural centrality of arboviral infections.

Methods: Strains of arboviruses were isolated on newborn white mice and in culture. Antigens of arboviruses and antibodies were found in ELISA and complement binding response.

Results: 123 strains of arboviruses of *tick-borne encephalitis, West Nile (WN), Batai, Tahyna, Tribec, Ukuniemi* were isolated from ticks, mosquites, small mammals, birds, patients with neuroinfections on newborn white mice. In observation of these objects in ELISA antigen of these viruses as well as viruses *Inko, Showshoe hare, Sindbis, Crimean hemorrhagic fever, hemorrhagic fever with kidney syndrome* were found in 166 cases. In Volyn, Lviv, Transcarpathian regions and in the Crimea active focuses with group occurrence of diseases among the people and high infection of ticks (6.3–19.5%), small mammals (8.3–15.9%) and presence of seropositive to tick-borne encephalitis people. Active focuses of WN virus which manifested themselves in group occurrence of diseases in people in Transcarpathian, Cherkasy and Kherson regions. Infection of mosquites in this focuses was 10.0–12.5%, and the number of seropositive to WN viruses people was 21.4–36.3%, Of the patients with acute season neuroinfections in 23.5% arboviral etiology was established by laboratory investigations.

Conclusions: The performed investigations testify to intensive circulation of arboviruses in Ukraine and their essential role in infectious pathology.

Vaccine and immunization

P1396 Cost-Effectiveness of Different Vaccination Strategies against Hepatitis B in Switzerland

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Objectives: To assess and compare the costs and epidemiological impact of different vaccination strategies against hepatitis B in Switzerland.

Methods: A birth cohort 85,000 individuals was followed over its lifetime using a decision tree analysis. Published data were used to simulate the risk of hepatitis B virus (HBV) infection in the cohort, the consecutive clinical outcomes and the associated costs. Five vaccination scenarios were assessed and compared to the baseline, defined as the current high-risk group strategy without prenatal screening. These were: 1. systematic prenatal screening and vaccination of newborns at risk; 2. universal vaccination of infants; 3. universal vaccination of schoolchildren; 4. universal vaccination of infants and schoolchildren; 5. universal vaccination of infants, schoolchildren and adolescents. Results are presented using a 3% annual discounting rate.

Results: Systematic prenatal screening reduced the number of chronic infections by 11% and prevented 6 deaths per year. The cost per year of life saved was estimated to be 23,350 CHF. The four universal vaccination scenarios had a much larger impact on the number of chronic infections and deaths prevented (reduction of 68–78%). Costs per year of life saved for universal vaccination ranged from 8820 CHF (infant strategy) to 12,380 CHF (schoolchildren strategy). However, the vaccination of schoolchildren would be as cost-effective as the vaccination of infants using alternative assumptions. 1. a lower compliance rate for infants compared to schoolchildren; 2. the need for a booster for infants.

Conclusion: Universal vaccination against hepatitis B is more cost-effective than the current selective vaccination strategy and would substantially reduce the number of chronic infections and deaths caused by HBV infections in Switzerland.

P1397 Clinical Trial of Simultaneous Administration of Recombinant Hepatitis B Vaccine and Tetanus Toxoid in Nonresponders

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Objectives: To compare the antibody responses to Pre S2 + S containing recombinant hepatitis B vaccine (S2SRHB) and S2SRHB + Tetanus toxoid (TT) in the nonresponders.

Methods: We assessed the efficacy of tetanus toxoid (TT) and Pre S2 + S containing recombinant hepatitis B vaccine (S2S RHB) in 10 nonresponders to S2SRHB. We applied simultaneous administration of S2SRHB and TT to ten persons (group I) who did not to respond (anti-HBs <10 IU/mL) after 3 doses of S2SRHB (given as 20 μgr/one dose, intramuscularly (i.m), in months: 0-1-2). 6 nonresponders (group II) received 3 additional doses by the same route as the initial vaccination (S2SRHB, i.m, 1 mo. Interval).

Results: In group I, after first S2SRHB +TT dose 2/10 (20%) responded with anti-HBs levels >10 IU/mL and after the second, another 3/10 (30%) responded and after the third, no more 0/10 (0%) responded, corresponding to a total response rate of 50%. In group II, after the first additional S2SRHB dose, 0/6 (0%) responded, with anti-HBS levels <10 IU/mL, after the second, 1/6 (16.66%) responded with anti-HBs level 15 IU/mL and after third dose no additional nonresponse was noted. Both seroconversion rate and the antibody titer level for anti-HBs antibody were significantly higher in group I than in group II ($p < 0.05$).

Conclusion: These results suggest that simultaneous administration of S2SRHB and TT is more effective in nonresponders than additional doses given by the same route as the initial vaccination.

P1398 Comparative Immune Response of Two Commercial Hepatitis B Vaccines

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Objectives: To describe results of a randomized, blinded trial comparing the immunogenicity and tolerability of an investigational; yeast recombinant Hepatitis B Virus (HBV) vaccine containing S antigen with a recombinant HBV vaccine containing pre S2 + S (from Chinese hamster ovary cell: CHO).

Methods: One hundred healthy, HBV seronegative persons were enrolled in a single blind randomized study to compare antibody and clinical responses to yeast recombinant S antigen vaccine (YSHBV) (Hepavax-gene, 1 mL: 20 μgr/one dose) and CHO recombinant Pre S2 + S vaccine (CS2SHBV) (Genhevac B, 0.5 mL: 20 μgr/one dose). 50 participiant (group I) received 20 μgr of YSHBV by intramuscular (i.m) injection at 0, 1 and 6 months. 50 partcipiants (group II) received 20 μgr of CS2SHBV by i.m injection at 0, 1 and 2 mo. serological and biochemical responses were measured at 0, 1, 2, 3, 6 and 7 months in both groups.

Results: The proportion of vaccines with minor local complaints (mainly local pain, myalgia) and proportion developing antibody to surface antigen (Anti-HBs) were similar for both vaccine groups ($p > 0.05$). But Anti-HBs titers were generally higher among recipients of CS2SHBV. Anti-HBs developed in 92% and 96%, in group I and II, at 7 month, respectively.

Conclusions: These data imply that YSHBV vaccine is as well tolerated and immunogenic as CS2SHBV vaccine. However, further studies should be necessary to compare duration of immunity at a long time points after vaccination for two vaccines.

P1399 Persistence of Anti-HBc Antibodies at Children Vaccinated against Hepatitis B

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Objectives: The long-term persistence of anti-HBc and the risk of hepatitis B infection at children vaccinated against hepatitis B were investigated.

Methods: The passive-active immunisation of 374 newborns against hepatitis B was started in the year 1988. All mothers were HBsAg positive at pregnancy, 19 of them were both HBsAg and HBeAg positive. The children received 50 IU of HBIG at birth and three 10-μg doses of plasma-derived or recombinant vaccine (H-B-vax, Engerix B) at 0, 1 and 6 months. The basic vaccination was finished at 331 children, 127 of them were revaccinated. Blood samples were tested by ELISA for anti-HBc (Sorin Biomedica, Italy) and for HBsAg, anti-HBs (Sevac, Czech Republic; Sorin Biomedica, Italy).

Results: Antibodies anti-HBc were checked at 347 children. Anti-HBc was found in 10 from 226 sera in the second year of their life, in 9 from 123 sera in the third, in 2 from 66 sera in the forth year and in 1 from 126 sera at older children. Only one boy was HBsAg positive and suffered hepatitis B. The new appearance of anti-HBc with increase of anti-HBs was observed at 1 child. New appearance of anti-HBc antibodies without increase of anti-HBs was described at 12 children, but 9 of them had only short persistence of anti-HBc. The increase of anti-HBs without revaccination was observed at 19 children.

Conclusion: Anti-HBc antibodies were found in 7.3% sera in the third year of life and 1.6% sera of elder children vaccinated against hepatitis B. In our study hepatitis B was described only at one child.

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P1400 Possibilities of Mass Vaccination against Viral Hepatitis B in the Czech Republic

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The universal vaccination against VHB is the only really effective way to fight the infection of hepatitis B virus as the methods of vaccination of risk groups cannot fully eliminate the disease.

The objective of our study was to judge the feasibility of introducing this measure in our local situation, especially in view of the current epidemiological situation and economical aspects.

Methods: We have tried to calculate the direct and indirect economical costs related to VHB in the Czech Republic in 1995. The total sum 100 mio Kc (4 mio USD) was compared in the van Damme model with a) vaccination cost of new-born mass vaccination by a DTP-HB tetravaccine, b) adolescent vaccination, c) vaccination of both these groups.

Results: In spite of descending trends and current incidence rate 6–7/100 000, prevalence of HBsAg varies between 0.5–0.67%. Based on the treatment data a qualified estimate was made of the direct costs over 30 mio Kc (1.2 mio USD) in 1 year and the indirect costs over 70 mio Kc (2.8 mio USD).

Conclusions: It is necessary to judge all the facts and in spite of high initial costs, to introduce the mass vaccination as recommended

by WHO. The experience from other countries (Italy, Germany, Poland, Spain, US) shows that this is the correct approach.

P1401 The Association between Measles Vaccine Response and HLA-DQA1 Alleles

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Background: The range of antibody (Ab) responses to measles vaccine (MV) virus may be explained by genetic diversity among the genes of the HLA cluster. As part of a large study, we sought to determine the role of HLA-DQA1 genes in predicting MV Ab response.

Methods: We determined the seroprevalence of measles Ab in 880 school children immunized with MMR-II at age 15 months. Ab levels were determined by a whole virus EIA Subjects who were non-responders (NRs) [n = 46] (IgG seronegative) and hyper-responders (HRs) [n = 68] (upper 10th percentile of IgG levels) were DQA1 typed using PCR-SSP. DQA1 allele frequencies and homozygosity rates were compared between the two groups.

Results: The allele frequency distribution between the NRs and HRs was significantly different ($p = 0.05$) with NRs having more DQA1*05 alleles ($p = 0.017$) and HRs having more DQA1*01 alleles ($p = 0.016$). The homozygosity rate among NRs was significantly higher than among HRs (21.7% vs. 8.8%, $p = 0.037$).

Conclusions: These data suggest an association between MV-induced Ab response and the distribution of DQA1 alleles. The presence of DQA1*05 alleles is associated with nonresponse and DQA1*01 alleles with hyper-response. In addition, DQA1 homozygosity is a significant a predictor of poor Ab response to measles vaccine.

P1402 Measles Revaccination Among Bolivian Children With Low or Absent Antibody Titre

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Objectives: To evaluate the response to revaccination among Bolivian children with low or absent measles antibody levels by haemagglutination inhibition (HI).

Methods: We revaccinated 202 children, aged 5–16 years, whose antibody levels were below 200 mIU by HI in a serosurvey conducted in April 1993 in Santa Cruz, Bolivia. A blood sample was collected immediately before vaccination (T0), 4 weeks later (T1), and 1 year after revaccination (T2) only from seroconverters. Sera were assayed by HI and by plaque reduction neutralisation assay (PRN). Sera collected 4 weeks post-vaccination were assayed for measles-specific IgM by enzyme-linked immunosorbent assay (EIA).

Results: Of the 202 revaccinated children, 148 (73%) showed high measles IgG levels at T0. A measles outbreak occurred in Santa Cruz about 6 months earlier. Of these 148, only 9 (6%) gave a history of measles since the 1993 survey. Of the 54 children with HI titres <500 at T0, 20 (37%) had no detectable HI antibody, and 34 (63%) had low antibody levels. Among 20 pre-revaccination seronegatives, 19 (95%) were seropositive at T1. None had a positive IgM response. Among 28 evaluable children with low HI antibody at T0, 12 (43%) had a fourfold increase at T1. At T2, 11 of 16 (69%) evaluable pre-revaccination seronegatives had a fourfold decrease in titre, and 3 had antibody levels <200 mIU. Among 12 pre-revaccination seropositives, 4 (33%) had a fourfold decrease, 6 a two-fold decrease, and 7 had levels <200 mIU. Among pre-revaccination seronegatives, the geometric mean titre (GMT) at T1 was 3933 by HI and 2062 by PRN. At T2, GMTs were 500 and 1093 by HI and PRN, respectively.

Discussion: No primary vaccine failures were detected. Children who responded to revaccination with a fourfold or greater rise in antibody titre should be considered as susceptible to reinfection. However, data from the recent outbreak suggest that the risk of developing symptomatic measles in these children is low. The persistence of antibody after revaccination was short.

P1403 Postvaccination Immunity Against Measles in Children, Residing on Radionuclide-Contaminated Territories of Belarus

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Objectives: To study the specific immunity against measles as well as to test the content of serum immunoglobulins classes G, A, and M in children, permanently exposed to low doses of ionizing radiation.

Methods: We examined 103 pre-school children from radionuclide-contaminated territories, immunized with a single dose of live measles vaccine, and 98 children from "non-contaminated" areas (controls) of the Republic. Specific measles antibodies were determined by means of passive hemagglutination assay, specific lymphocyte proliferative activity was measured in the reaction of lymphocyte blasttransformation with antigens of measles vaccine, and serum immunoglobulin concentrations were analyzed by radial immunodiffusion technique.

Results: Reliably lower lymphocyte proliferative activity to antigens of measles vaccine was registered among children, residing on radionuclide-contaminated territories. Besides, we identified the decrease of antibody-mediated measles immunity: the percent of seropo-sitive children constituted 68.4% versus 98.0% in controls ($p < 0.05$), and geometric mean titers of hemagglutinin-antibodies made up 1.80 Ig versus 1.35 Ig ($p < 0.05$). Changes in specific postvaccination immunity were observed on the background of disbalance in serum immunoglobulin production: IgG concentration was lower than in controls (6.37 ± 0.38 g/l and 8.26 ± 0.40 g/l, respectively, $p < 0.05$), but IgM concentration was higher (1.23 ± 0.16 g/l and 0.77 ± 0.04 g/l, respectively, $p < 0.01$).

Conclusions: The detected deviations in the children's immunity allow one to consider the radionuclide-contaminated territories as risk areas of disturbances in immunity response, including postvaccination immunity.

P1404 Immunity to Measles-, Mumps-, Rubella-, and Polioviruses in Medical Students at the University of Bern, 1996

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Objectives: To identify medical students lacking antibodies to measles-, mumps-, rubella- and polioviruses, and to offer them vaccination.

Methods: IgG antibodies to measles-, mumps-, and rubellaviruses in the sera of 141 students sera were determined by commercially available ELISA assays. Sera from 130 students were tested for neutralizing antibodies to polioviruses types 1–3 using a modified microplate neutralization assay. Students lacking antibodies to at least one of those viruses were contacted and offered to receive the respective vaccine.

Results: Four male students lacked antibodies to rubellavirus. Evidence of immunity to measles- and mumpsvirus was absent in 9 (f:m = 4:5) and 18 (f:m = 8:10) subjects, respectively, and one male student had no IgG to both measles- and mumpsvirus. 31 of those 32 students were eventually vaccinated with the MMR vaccine.

Only one female student lacked neutralizing antibodies to polioviruses 1, 2 and 3. She had never received polio vaccination and a primary course of this vaccine was recommended.

Conclusion: The determination of antibodies to measles-, mumps-, and rubellaviruses in medical students in order to vaccinate subjects lacking immunity seems justified with respect to the potential risk of being exposed to patients and of being the source of a preventable infection for their future patients.

P1405 Measles Immunity After Vaccination: Results in Children Vaccinated Before 12 Months of Age

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Objectives: We analyzed serum samples of children between the ages 4–6 years who had been vaccinated before 12 months of age to evaluate measles seropositivity.

Methods: Sera collected from 140 children between the ages 4–6 years who were selected by random sampling procedure in a squatter area, Antalya, Turkey. The children had been vaccinated before 12 months of age and had not received booster dose. Anti-measles IgG antibodies were assayed by ELISA method.

Results: Anti-measles IgG was detected in 122 of 140 (87%) serum samples of children.

Conclusion: We found a high rate of measles seropositivity in children between the ages 4–6 years who had been vaccinated before 12 months of age and who had not received booster dose. Further studies are needed to define the timing of booster dose for children of this region.

P1406 Safety and Immunogenicity of a New DTPa-HBV-HIB Combination Vaccine for Primary Immunisation of Infants

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Objectives: To compare the safety and immune response in infants vaccinated with 3 doses of PRP-tetanus conjugate mixed with a combined DTPa-HBV vaccine to a control group that received separate injections of DTPa-HBV and PRP-tetanus conjugate in opposite limbs.

Methods: 487 infants were vaccinated at 3, 4 and 5 months of age and a booster consisting of unconjugated PRP was administered separately or mixed with DTPa-HBV in the second year of life. Antibody concentrations were measured before and after primary vaccination and booster dose.

Results: The primary course of tetanus-PRP elicited a good humoral immune response, 95.5% of all subjects having seroprotective anti-PRP concentrations ≥ 0.15 μg/ml. by the time of the booster, this proportion had declined to 81.2% in the groups given separate DTPa-HBV and Hib vaccines and to 47.8% in the groups given mixed DTPa-HBV/Hib vaccines. However, the booster elicited a marked and rapid increase in anti-PRP-antibodies in all groups. Antibody concentrations >1.0 μg/ml, a value associated with long-term protection, occurred in 86.4–97.9% of the vaccinees. The DTPa-HBV components elicited protective antidiphtheria, anti-tetanus (>0.1 IU/ml) and anti-HBs antibody levels (>10 mIU/ml) in >98.5% of the infants. Mixture of these antigens with Hib in one syringe did not influence immune response to these antigens.

Conclusions: Although there seems to be interference of the Hib-vaccine after primary vaccination (low titres), the antibody response to the booster indicates effective priming with the combination vaccine.

P1407 Evidence for Polysaccharide-Specific B-Cell-Memory in the First Year of Life: Plain Hib-PRP as a Booster after Priming with a TT-Conjugate Hib-Vaccine

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Objectives: To investigate whether conjugated-PRP-vaccines induce polysaccharide-specific B-cell-memory in the first year of life.

Methods: We examined the immune response to a dose of *unconjugated* Polyribosyl-ribitol-phosphate (PRP) administered in the second year of life to children primed with three doses of PRP-tetanus conjugate reconstituted with a diphtheria-tetanus-acellular pertussis-hepatitis B vaccine (DTPa-HBV) at 3, 4 and 5 months of age. A measurable immune response to a booster dose of *plain* PRP should provide evidence for the existence of immunological memory from a primary vaccination course.

Results: The *unconjugated PRP* elicited protective anti-PRP antibody levels (≥ 0.15 μg/ml) in all but 3 of the 369 vaccinees, including 13 infants who failed to demonstrate a measurable immune response after primary course. In a sub-cohort of 54 subjects all had anti-PRP levels ≥ 0.5 μg/ml within 7–14 days of the booster showing a rapid anamnestic type response. Both primary and booster responses were predominantly IgG 1 indicating a T-cell dependent response.

Conclusions: For the first time we prove the induction of polysaccharide specific B-cell-memory by a PRP-tetanus vaccine when administered simultaneously with a candidate DTPa-HBV-vaccine in the first 18 months of life. Successful induction of immunological memory occurred even when there was no measurable humoral anti-PRP response to the primary course.

P1408 Immunogenicity of the Acellular Pertussis Vaccine Combined with Diphtheria and Tetanus Toxoids (DTacP) and *Haemophilus influenzae* Type B and Tetanus Toxoid (PRP-T) Conjugated Vaccine in Mexican Children at 18 Months of Age

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Objective: To compare the immunogenicity of the five-component DTacP vaccine and PRP-T vaccine, administered combined (A) or separate (B) vs. the immunogenicity of the whole cell pertusis vaccine (DPT) and PRP-T administered combined (C).

Methods: Double blinded, randomized, controlled study, in 102 children who had received primary immunization with DPT. Immunogenicity was assessed by measurements of antibodies to all vaccine antigens.

Results: Immune response to all antigens in A, B and C groups was excellent. This response (GMT) for Diphtheria and Tetanus, PRP and Pertussis agglutinins was comparable in all three groups. PT, FHA, FIM and Pertactin responses were higher in A and B than in C ($p \leq 0.02$ for PT and Pertactin). Both DTacP groups (A and B) had comparable responses. Protective titres (≥0.1 IU/ml) for Diphtheria and Tetanus were present in 73–92% of children pre and in 100% post-immunization. Protective titres for PRP (≥0.15 ug/dl) in 81% of children pre and in 100% post-immunization.

Conclusion: The immune response, in children who received the five-component DTacP and PRP-T combined or separate, was comparable. Pertussis antibody responses were higher in children receiving DTacP than those who received DPT. Immunogenicity to PRP-T did not change when it was reconstituted with DTacP or DPT. This study indicates that Mexican children primed with three doses of DPT could receive a booster with the five-component DTacP to reconstitute PRP-T.

P1409 RCT of Ibuprofen vs. Acetaminophen for Preventing Fever from Whole-Cell-Pertussis-Containing DTP Vaccination

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Objective: To determine the efficacy of ibuprofen versus acetaminophen in fever prevention in the 48 hours after whole-cell pertussis-containing DTP vaccination.

Methods: In this double-blind trial, we randomized healthy subjects aged 6–18 months due for routine DTP-Hib or DTP vaccinations to receive post-vaccination either ibuprofen (9.5 mg/kg) at 0 and 8 hours (placebo at 4 hours) or acetaminophen (15 mg/kg) at 0, 4, and 8 hours. The child's parent measured fever (rectal temperature > 38°C) and other adverse reactions during the next 48 hours.

Results: Of the 306 enrolled, 52% were male, and 91% Caucasian. 155 received ibuprofen, and 151 acetaminophen. The 2 groups were comparable in distribution of gender, race, vaccination status, dose of DTP-containing vaccine due, age, and weight. 44% had fever with ibuprofen, while only 33% had fever with acetaminophen ($p = 0.051$). Using maximum temperature (Tmax) measured over the following 48 hours, the mean Tmax was 38°C for ibuprofen and 37.7°F for acetaminophen ($p < 0.01$). The rate of any adverse reaction was similar – ibuprofen, 88%, and acetaminophen, 90%. The rate of moderate-severe reactions was 55% with ibuprofen but only 39% with acetaminophen ($p < 0.01$).

Conclusions: Ibuprofen did no better than acetaminophen in preventing fever from whole cell pertussis containing DTP vaccination. The rates were the same and in fact the Tmax was higher with ibuprofen. Furthermore, ibuprofen appears to result in higher rates of moderate-severe adverse reactions.

P1410 Immunization of Adults Against Diphtheria in Poland

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Objectives: Immunological response of Td vaccine in adults.

Methods: Three groups of adult volunteers received one dose of Td vaccine: 51 students, aged 19 to 24 years, 40 adult from Warsaw (25–65), 44 adult from Krakow (22–57). Tetanus-diphtheria vaccine with a reduced amount of diphtheria toxoid (2Lf per dose) has been produced locally since 1993. Blood samples were taken just before and 4 weeks after immunization, and the level of diphtheria antibodies was determined by passive haemagglutination test. 0.1 IU/ml was considered as a protective level.

Results: Td vaccine was well tolerated, slight and temporary reactions were reported 1–2 days after immunization and disappeared after 2–3 days. 66% of volunteers was protected against diphtheria before immunization. The group aged 19 to 24 years showed a higher level of immunity than the oldest. after vaccination the geometric mean of diphtheria antibody titters increased significantly in the people who had been previously vaccinated against diphtheria. Volunteers with moderate levels of protective antibodies were reimmunised with monovalent diphtheria toxoid "d".

Conclusions: One booster dose of Td vaccine stimulated o good production of diphtheria antibody.

P1411 Vaccination with Pertussis Toxoid Decreases Spread of Pertussis within the Family

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Background: General vaccination will not only protect the vaccinees, but may also induce herd immunity.

Methods: During a double-blind, placebo-controlled trial of an acellular pertussis toxoid vaccine (developed at NIH, USA; manufactured by AMVAX, USA) the risk of pertussis was investigated in parents and younger siblings of 3450 vaccinees, randomized to receive DT toxoids with or without pertussis toxoid at 3, 5 and 12 months of age. During 2 years after the 3rd vaccination pertussis cases were actively diagnosed in *all* family members (N = 11,000).

Results: Pertussis with paroxysmal cough ≥21 days (definition of WHO) was diagnosed in 11 DTP parents and in 26 DT parents; indirect protection 60 percent (*95% CI:* 16–82%). In younger siblings there were 10 WHO cases in the DTP group and 18 cases in the DT group; indirect protection of 43% (−1–76%). When cases of pertussis with cough ≥7 days were included, indirect protection was 44% (7–67%) in parents and 56% (9–81%) in younger siblings.

Conclusion: Vaccination of children with pertussis toxoid decreases the risk of pertussis in close contacts, basis for the induction of herd immunity.

P1412 Kinetics of Humoral Immune Response to Hemagglutinin and Neuraminidase After Vaccination with Split Influenza Vaccine

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Objectives: The aim of this study was to evaluate the kinetics of hemagglutination inhibition (HI) and neuraminidase inhibition (NI) antibodies after vaccination against influenza in the epidemic season 1993/1994.

Methods: One hundred and eighty-eight volunteers in four different age groups (young people, adults, middle-aged people, the elderly) were immunized with trivalent split influenza vaccine (Vaxigrip, Pasteur-Merieux) and compared with a control group of 108 volunteers of the same age. Antibodies were determined in pre-vaccination sera and three week and six month post-vaccination sera. The hemagglutination inhibition (HI) test and neuraminidase inhibition (NI) test were performed by a routine technique for viruses recommended by the WHO for the epidemic season 1993/1994.

Results: Mean fold antibody increase was highest for the component H1 (17.9), medium for H3 (6.2) and lowest for the component HB (3.5). The mean fold antibody increase for neuraminidase was

highest for the component NB (13.8), lower for N2 (2.97) and lowest for the component N1 (2.6).

Conclusions: Antibody responses measured by hemagglutination and neuraminidase inhibition titration showed different kinetic patterns. Geometric mean titres for hemagglutinin and neuraminidase were notably higher six months after vaccination than before vaccination.

P1413 Influenza Immunization: Improving Compliance of Health Care Workers (HCW)

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Objective: In spite of yearly recalls, influenza immunization rates of HCW remained low (<10%) in HUG. This study was conducted to 1) identify HCW reasons for rejection of immunization, 2) design specific intervention methods based on these reasons and 3) evaluate the impact of such interventions.

Methods: 3 departments with high-risk patients (geriatrics, obstetrics and pediatrics) were selected as main targets and questionnaires were distributed in these units. Based on HCWs' perceptions, we employed 4 intervention methods to increase compliance: (1) educational conferences; (2) posters ("talking walls"); (3) personal letters; (4) on-site availability of a vaccination nurse. Immunization rates were collected throughout the institution.

Results: 797/1300 (61%) questionnaires were received and the main reasons for immunization rejection identified. During the 8-week period after start of the promotion campagne, 1441 (26%) of 5432 HCW were vaccinated in HUG. In the 3 selected departments with high-risk patients, influenza immunization rates rose dramatically (from <10% to 37%). Nurses were more reluctant to immunization compared to other HCW.

Conclusions: Influenza immunization rates of HCW can be increased by specific interventions among which the on-site availability of a vaccination nurse appeared essential.

P1414 Development of a *Salmonella*-based Prophylactic Vaccine Against Human Papillomavirus HPV 16

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The induction of neutralizing antibodies in genital secretions may be critical for the effectiveness of a prophylactic vaccine against genital human papillomaviruses (HPV), which are associated with the development of cervical cancer. Attenuated strains of *Salmonella* are attractive live vaccine candidates for eliciting mucosal as well as systemic immune responses. The L1 major capsid protein of HPV16 has been expressed in the PhoP^c strain of *S. typhimurium* in which it self-assemble into virus-like particles (VLPs) that resemble authentic HPV virions. BALB/c mice were immunized with the HPV16 L1 recombinant PhoP^c strain by the oral and nasal route. Despite a low stability of the L1-expressing plasmid *in vivo*, a double nasal immunization was effective in inducing L1-specific antibodies in serum. These antibodies recognized native VLPs and effectively neutralized HPV16 pseudotyped virions in an *in vitro* infectivity assay. Anti-VLP IgA and IgG were also detected in oral and vaginal secretions, indicating that potentially protective antibodies were elicited at these sites. Recombinant *Salmonella* expressing HPV capsids are a promising vaccine candidate against genital HPV infection and we are currently testing new strains that maintain the plasmids and could be used in human.

P1415 Creation of *Salmonella enteritidis* Mutant with Stable Avirulent Phenotype

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Objectives: Though salmonellosis remains one of the actual problems in medicine and veterinology the creation of attenuated *Salmonella* strains is actually. Attenuated *Salmonella* strains are also suitable as delivery-strains of heterologous antigens. As species *Salmonella enteritidis* is the main causative agent of salmonellosis in Russia, creation of attenuated mutant of this species will be perspective, as such mutant could be used as vaccine strain in veterinology or delivery-strain.

Methods: Mutant was obtained from the strain of the wild type *S. enteritidis 1791* during processes of transduction and next direct selection. Created mutant, named *S. enteritidis E-23* was determinated as cya^s (cya-suppressor) mutant, because E-23 possesses cya (lacing adenilatecyclase) mutation with CRP-phenotype (lacing c-AMP-receptor protein). Mutant E-23 didn't possess any antibiotic resistance.

Results: 1 g LD 50 of E-23 −8.5; 1 g LD 50 of parent strain −1 after intraperitoneally inoculation of mice. Mutant E-23 possessed of stable avirulent phenotype: 1 g LD 50–9 after 8 passing through mice organism. Mutant E-23 has persisted in mice during 45 days after single oral application. No secretion of the mutant and no any change in mice enteric microflora during period of persistence was observed. Mutant E-23 didn't differ stable phenotype and other properties after deep cultivation and preparation of dry vaccine form.

Conclusion: Cya s mutant of *S. enteritidis* with stable avirulent phenotype and capability to persist in mice after single oral application was obtained.

P1416 PCR System for Identification of crp Mutation in *Salmonella*

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Objectives: Elaboration of the new generation of recombinants vaccines for oral application is one of the perspective scientific trend in modern microbiology. Attenuated *Salmonella* strains have received the most attention as perspective carries of heterologous antigens. Cya and crp mutants (lacing adenylatecyclase and c-AMP receptor protein, respectivity) of *Salmonella* have been received the acknowledgment among a attenuated *Salmonella*. The methods of genetic's recombination by transduction and transformation are preferable for creation such mutants. For this purpose we decided to create donor's strain, possessing genetic's marking Δcrp mutation and laboratory investigating system for identification such mutation.

Methods: Donor's strain *S. typhimurium B-5* have been obtained by treatment with phage *P22* (Tn10) and next exclusion of Tn10. The Δcrp mutation was estimated by determination of nucleotide sequence. PCR method was used for determination of Δcrp mutation.

Results: Mutant *S. typhimurium B-5* possessed deletion with 7 nucleotides in crp gene. Δcrp gene has been transducted to other wild type strains of different species of *Salmonella*. For determination Δcrp mutation the special primer's, complementary Δcrp gene were elaborated.

Conclusion: *Salmonella* mutant with deletion of 7 nucleotides in crp gene was obtained. The special PCR-system for determination such mutation was created.

P1417 Antibody Response to *Campylobacter* Antigens in Immunized Mice

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Objective: Evaluation of *Campylobacter jejuni* and *coli* antigenic components which elicit an immune response and identification of classes of antibodies stimulated.

Methods: Five *C. jejuni* and four *coli* strains representing a range of Lior serogroups and flagellar gene polymorphism groups were used to intranasally challenge nine groups of Balb/c mice. Antigenic components of each strain were determined by SDS-PAGE and immunoblotting of bacterial cell onicates (CS) and acid-glycine extracts (AGE) with immune mouse serum.

Results: Protein profiles of CS and AGE showed heterogeneity of flagellar proteins (molecular mass (M_r) range 58k to 61k) and differences in M_r of these and other cellular components. Immunoblotting of CS and AGE with homologous and heterologous mouse serum demonstrated IgG, IgA and IgM reactions to at least one flagellar protein, but only IgA and IgG reactions to outer membrane proteins (OMP, M_r 20k and 25k) and other cellular components (M_r 40k and 45k) of CS. Immunoblotting of CS with IgG subclasses showed that flagellar components were recognized by IgG_1, IgG_2 and IgG_3 antibodies whereas OMP and other components were only recognized by IgG_1 and IgG_2.

Conclusions: Strain differences observed in protein profiles, immunogenicity and relative antigenicity of protein components were not related to Lior serogroup or polymorphic group differences. In all strains examined, flagellar proteins showed higher cross-reactivity and stimulated all classes and subclasses of antibodies, suggesting their involvement in all aspects of host defense, induction of cytotoxicity and in protective immunity.

P1418 Analysis of *Corynebacterium diphtheriae* Toxin IgG Antibody in Sera of Healthy Population in Poland

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Diphtheria is an endemic disease in many regions of the world due mainly to inadequate health-care delivery systems. The recent outbreaks of diphtheria in Belarus, the Russian Federation and Ukraine poses a threat to neighbouring countries, including Poland. An increased incidence of diphtheriae has already been reported in this country. The aim of this study was to standardise an ELISA for the determination of specific IgG against *C. diphtheriae* toxin, in order to measure the protective antibody level in the sera of healthy people. The normal level of antibodies against diphtheria toxin in over 400 serum samples from healthy population in different age groups (10 samples each in intervals of one month over the first year of life and then 10 samples each in consecutive years) and adequate diphtheria immunisation status was determined. The optimum antigen coating dose – 2.5 Lf of diphtheria anatoxin per ml of carbonate buffer pH 9.6 was estimated. The optimum dilutions for specific IgG determination for serum samples and the peroxidase conjugate were defined to be 1:100 and 1:5000 respectively. Antigen–antibody incubation and conjugate treatment were performed for 1 hour at 37°C. The British NBSB diphtheria antitoxin human serum was used as a reference. The obtained results of analysed sera were calculated according to standard curve. Consecutive vaccination increases anti-diphtheria IgG level, what was demonstrated by geometric mean titer calculation. The protective antibody level (0.1 JA/ml) in analysed samples was calculated for each particular age group. The lack of significant protective antibody level was observed in some sera samples of the adult group.

P1419 The Effect of Immunization with "Pervalur"-Torlak (polyvalent urovaccine)

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Objectives: "Prevalur"-Torlak is polyvalent vaccine with inactivated uropathogenic bacteria. It consists of 7 bacterial strains in appropriate concetration. Immunological response and protective efficacy of this vaccine have been examinated.

Methods: Active immunization have been applied in 63 persons, mostly female, age 21 to 71 with recurrent urinary tract infection. Vaccine was given subcutaneously 3 doses at weekly intervals and 4th one month after the 3rd. The titres of specific circulatory antibodies were followed on days 0., 21. and 45. by agglutination test and total amount of IgG, IgA and IgM in serum were determined by RID method. All patients were examined during twelve months.

Results: "Prevalur"-Torlak induces formation of specific antibodies and the antibody titres increased 4-fold and more in all patients after 21th day (mean 1:2.88.39) and 45th day (1:681.39) compared to 0th day (1:44.17). This immunization led to significantly higher serum IgG and IgA ($p < 0.001$) in 48 persons. In 26 patients frequency of recurrent UTI was reduced from 1 episoda/1.5 patient months to 1 episoda/6.3 patientmonths, and 38.09% of patients were without infection during the period of examination.

Conclusions: Immunization with "Prevalur"-Torlak induces the formation of specific antibodies in significant titres in all patients and increases of total amount of serum IgG and IgA in 76.19%. The protective effect in majority of patients leads to conclusion that polyvalent vaccine might be applied safely to the patients suffering from UTI.

Herpes viruses (HSV, VZV, EBV and HHV-6)

P1420 Susceptibility of Herpes Simplex Virus (HSV) to Acyclovir: Absence of Correlation between Results Obtained in Vero and MRC-5 Cell Lines

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Objectives: To compare the susceptibility of clinical isolates of HSV to acyclovir (ACV) on Vero and MRC-5 cells and to evaluate its clinical correlation.

Methods: Susceptibility testing was performed by the "standard" method of plaque reduction assay following the American Society for Microbiology guidelines on Vero cells as well as on MRC-5 cells. We considered a strain resistant when the IC_{50} for ACV was greater than 2 μg/ml. Three control strains were used, one susceptible to ACV, one resistant to ACV and one resistant to ACV and foscarnet.

Results: Among the 33 isolates of HSV, 21 were susceptible to ACV on both cell lines. Twelve isolates were resistant to ACV on Vero cells, among them, five were resistant and seven susceptible